Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/IJMYCO

Short Communication

Stratifying low level Isoniazid resistance using additional intermediate drug concentration

Rajagopalan Lakshmi [a], *Ranjani Ramachandran* [b], *A. Syam Sundar* [a], *Fathima Rahman* [c], *Vanaja Kumar* [a,*]

[a] *Department of Bacteriology, National Institute for Research in Tuberculosis (Formerly Tuberculosis Research Centre), Chetput, Chennai 600 031, India*

[b] *World Health Organization – India Office, New Delhi, India*

[c] *Department of Statistics, National Institute for Research in Tuberculosis (Formerly Tuberculosis Research Centre), Chetput, Chennai 600 031, India*

ARTICLE INFO

Article history:
Received 19 December 2013
Received in revised form
5 February 2014
Accepted 6 February 2014
Available online 12 March 2014

Keywords:
M. tuberculosis
Minimum inhibitory concentration
Isoniazid
Drug resistance

ABSTRACT

Isoniazid (INH) susceptibility testing for 100 *Mycobacterium tuberculosis* performed by conventional minimum inhibitory concentration (MIC) method was stratified using additional drug concentrations. Introduction of additional drug concentrations did not greatly improve the discriminatory capacity, but can be used in specialized studies pertaining to cross resistance between structural analogues of INH.

Isoniazid (INH) is considered a vital drug in the treatment of tuberculosis (TB) [1]. The mechanism of INH action on *Mycobacterium tuberculosis* is narrowed down to mycolic acid synthesis in the cell wall. Molecular studies on INH indicated the involvement of *kat*G and *inh*A genes playing a major role in resistance [2]. Phenotypic and genotypic methods for drug susceptibility testing (DST) have been well evaluated for INH. Similarity in structure and cellular target of INH and ethionamide (ETO) has led to the phenomenon of cross resistance between these drugs. It is reported that low level INH resistant isolates are likely to be ETO resistant [3–5]. An earlier attempt to identify cross resistance between these drugs indicated the need for revisiting DST for INH [6]. Though the conventional minimum inhibitory concentration (MIC) method on solid Lowenstein-Jensen (LJ) medium for INH is a time-tested procedure, there lies a vast difference between the drug concentrations used for defining the susceptibility profile [7]. To classify the low level of INH resistance more precisely, susceptibility testing was carried out using drug concentrations ranging in-between the existing concentrations used in the MIC method.

* Corresponding author. Present address: Centre for Drug Discovery and Development (3D), Sathyabama University, Jeppiaar Nagar, Rajiv Gandhi Salai, Chennai 600 119, India. Tel.: +91 24503150/51/52/54/55x5693.
E-mail address: vanaja_kumar51@yahoo.co.in (V. Kumar).

http://dx.doi.org/10.1016/j.ijmyco.2014.02.001

Table 1 – Comparison of susceptibility of INH by conventional and modified MIC methods.

Modified MIC method	Conventional MIC method			
	Susceptible (at MIC ⩽0.2 μg/ml)	Low level Resistant (at MIC 1.0 μg/ml)	Resistant (at MIC ⩾5.0 μg/ml)	Total
⩽0.2 μg/ml	44	2	2	48
0.5 μg/ml	1	7	0	8
1.0 μg/ml	2	4	3	9
3.0 μg/ml	0	0	6	6
⩾5.0 μg/ml	0	0	29	29
Total	47	13	40	100

Drug susceptibility testing for INH in 100 *M. tuberculosis* isolates was performed by the conventional MIC method following standard operating procedures [8]. In addition to the existing INH concentrations, 0.2, 1.0 and 5.0 μg/ml, and two more intermediary INH concentrations, namely 0.5 and 3.0 μg/ml, were introduced as a modification, and the isolates were subjected to retesting by the modified method. Standard interpretation was followed for conventional MIC method and isolates with an MIC of 0.5 μg/ml were considered as susceptible and 3.0 μg/ml as resistant, respectively.

Among the 100 *M. tuberculosis* isolates analyzed by conventional MIC method, 47% and 40% were susceptible and resistant towards INH. Borderline resistance (MIC of 1.0 μg/ml) was observed in 13% of the isolates. Including additional INH concentration in modified MIC method, 48% and 29% showed susceptible and resistant profile (Table 1). Borderline resistance with MIC of 1.0 μg/ml was observed with 9% of isolates. MIC of 0.5 μg/ml and 3.0 μg/ml was exhibited by 8% and 6% of the isolates, respectively. Comparison of both the MIC methods indicated that 7 of 13 borderline isolates (with MIC of 1.0 μg/ml) in conventional MIC method exhibited a MIC of 0.5 μg/ml by modified MIC method. Among the 47 susceptible isolates by conventional MIC method, only 3 were discrepant by the modified method; wherein, a single isolate had a MIC of 0.5 μg/ml, and 2 showed borderline resistance with a MIC of 1.0 μg/ml. Within the resistant category in conventional MIC method, 3 and 2 isolates exhibited borderline resistant and susceptible phenotypes by modified MIC method, and 6 isolates had a MIC of 3.0 μg/ml by modified MIC method.

Resistance to INH has become a frequent observation among all drug resistant clinical isolates, with incidence as high as 20% to 30% in some regions [9]. Phenotypic DST in solid LJ medium and in MGIT 960 liquid culture systems are routinely used in laboratories across the world for the detection of INH resistance [10]. An attempt to detect low level INH resistance (phenotypic) was novel and was hypothesized to provide substantial evidence for cross-resistance between INH and ETO. With the introduction of additional INH concentrations, it was evident that isolates exhibiting borderline resistance are tricky to interpret as they may indicate either of the phenotypes upon repeat testing. None of the borderline isolates upon retesting showed a MIC of 3.0 μg/ml, again reiterating the fact that borderline isolates have a tendency to shift towards susceptible phenotype. Another explanation could be that the ratio of resistant and susceptible isolates should be near equal, and phenotypes of the dominant clones are portrayed. Presence of borderline phenotypes among the susceptible isolates could also be due to the above phenomenon. The two resistant isolates that showed susceptible phenotype by modified method had low colony counts, and the discrepancy could be due to technical error.

Overall, the existing INH concentrations used in MIC method can be continued as standard format for INH susceptibility testing. The attempt to classify low level INH resistance using additional INH concentrations though could not completely distinguish borderline INH resistant isolates; it had substantially classified a large proportion of them as susceptible. Validity of these results can be confirmed by molecular identification. Modified MIC method for the detection of low level INH resistance could be used only to resolve the results obtained in studies on cross-resistance between INH and its structural analogues.

Conflict of interest

As the authors of the manuscript, we do not have a direct financial relation with the commercial identities mentioned in the manuscript that might lead to a conflict of interest for any of the authors.

Acknowledgements

The authors wish to thank the financial assistance provided by WHO through NIH/USAID and ICMR for infrastructure facilities. One of the authors, RL wishes to thank ICMR & WHO for providing financial assistance. The authors would like to thank the Staff, Department of Bacteriology for their technical support.

REFERENCES

[1] L.C. Santos, The molecular basis of resistance in *Mycobacterium tuberculosis*, Open J. Med. Microbiol. 2 (2009) 24–36.

[2] A. Banerjee, E. Dubnau, A. Quemard, V. Balasubramanian, K.S. Um, T. Wilson, et al, inhA, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*, Science 263 (1994) 227–230.

[3] A. Quemard, G. Laneelle, C. Lacave, Mycolic acid synthesis: a target for ethionamide in mycobacteria?, Antimicrob Agents Chemother. 36 (1992) 1316–1321.

[4] G.P. Morlock, B. Metchock, D. Sikes, J.T. Crawford, R.C. Cooksey, ethA, inhA, and katG loci of ethionamide-resistant clinical *Mycobacterium tuberculosis* isolates, Antimicrob. Agents Chemother. 47 (2003) 3799–3805.
[5] Y.M. Zhang, S.W. White, C.O. Rock, Inhibiting bacterial fatty acid synthesis, J. Biol. Chem. 281 (2006) 17541–17544.
[6] R. Lakshmi, V. Kumar, M. Baskaran, A.S. Sundar, F. Rahman, N. Selvakumar, et al, Pattern of ethionamide susceptibility and its association with isoniazid resistance among previously treated tuberculosis patients from India, Braz. J. Infect. Dis. 15 (2011) 219–220.
[7] G. Canetti, W. Fox, A. Khomenko, H.T. Mahler, N.K. Menon, D.A. Mitchison, et al, Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes, Bull. World Health Organ. 41 (1969) 21–43.
[8] Standard Operating Procedure for Mycobacteriology Laboratory. Department of Bacteriology, National Institute for Research in Tuberculosis (NIRT), ICMR, Chetput, Chennai 600 031, India. Version 1.1, November 2010, pp. 86–96.
[9] S. Siddiqi, P. Takhar, C. Baldeviano, W. Glover, Y. Zhang, Isoniazid induces its own resistance in nonreplicating *Mycobacterium tuberculosis*, Antimicrob. Agents Chemother. 51 (2007) 2100–2104.
[10] C. Piersimoni, A. Olivieri, L. Benacchio, C. Scarparo, Current perspective on drug susceptibility testing of *Mycobacterium tuberculosis* complex: the automated nonradiometric systems, J. Clin. Microbiol. 44 (2006) 20–28.